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RESTRICTED CYTOKINE EXPRESSION IN RHEUMATOID ARTHRITIS

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Objective. To determine the cytokine profile of the phenotypically activated T cell in rheumatoid arthritis (RA) synovium.

Methods. Interleukin-2 (IL-2), IL-2 receptor (IL-2R), IL-6, IL-4, and interferon-γ (IFNγ) gene expression was examined in T cells from freshly isolated synovial fluids (SF) and synovial tissues (ST) from patients with RA. Estimates of baseline expression were determined using unstimulated peripheral blood (PB) T cells from healthy individuals. The corresponding positive controls were phytohemagglutinin-activated tonsil T cells.

Results. In studies of paired PB and SF T cell samples from 17 RA patients, IL-2 messenger RNA (mRNA) levels in only 1 PB and 3 SF samples were more than 2 standard deviations above the mean of levels in unstimulated PB from healthy donors. Similarly, only 5 PB and 7 SF samples exhibited elevated IL-2R mRNA levels. IFNγ gene expression was not detected in any of the paired RA PB or SF samples. Fractionated T cells from 12 RA ST were screened with similar results: Only 1 of 12 samples exhibited IL-2 mRNA levels more than 2 standard deviations above levels in baseline controls. IL-2R mRNA levels were low or not detected, and IFNγ mRNA was absent. Subsequent studies showed that IL-4 and IL-6 gene expression levels were also low in RA tissues compared with tonsil T cell–positive controls.

Conclusion. These data provide evidence for restricted cytokine expression in the T cell population in RA tissues.

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic, destructive and inflammatory lesions of synovial joints (1). In this chronic inflammation, the importance of T cell functions, such as cytokine release, is unclear. Several cytokines that promote synovial cell proliferation have been implicated in the development of membrane hyperplasia and pannus formation in RA. Synoviocyte collagenase and proteoglycan production, synovial cell proliferation, and granulocyte–macrophage colony-stimulating factor (GM-CSF) production are enhanced by tumor necrosis factor α (TNFα) and interleukin-1 (IL-1) (2–6); collagen production by fibroblasts is induced by transforming growth factor β (TGFβ) (7); IL-2 and IL-6 are T cell growth factors implicated in influencing autoantibody production (8,9). The phenotypic evidence of T cell activation in rheumatoid synovium (10–13) suggests a role for T cell–secreted cytokines in mediating matrix destruction to the synovium. The objective of the present study was to examine the cytokine profile in synovial T cells from patients with RA, with reference to IL-2, IL-4, IL-6, and interferon-γ (IFNγ).

Cytokines exert their effects in a coordinate manner; thus, any cytokine imbalance may serve to drive or perpetuate the inflammatory process. Im-

This work is dedicated to the memory of Rachel Lachs.

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Supported by grants from The Arthritis Society of Canada and the Medical Research Council of Canada. Dr. Fish is the recipient of an Ontario Ministry of Health Career Scientist Award.

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Submitted for publication March 6, 1992; accepted in revised form January 24, 1993.

paired production of specific T cell–secreted cytokines would affect interactions with other regulatory factors. For example, the mutual antagonism between IFNγ and TNFα, with respect to HLA–DR expression, cell proliferation, collagenase production, and GM-CSF production, allows for coordinate regulation of specific processes (2). Any imbalance in either cytokine would affect this regulation, specifically in the rheumatoid synovium. Many of the proliferative and stimulatory activities of IL-4 are down-regulated by IFNγ (14,15). TGFβ is inhibitory with respect to IL-2, IL-4, and IL-6 production (16,17) and reduces IFNγ-induced HLA–DR expression in human cells (18). Thus, the cytokine profile in RA synovium may provide specific insights into dysregulated processes that perpetuate the persistent synovial inflammation.

In recent years a number of different approaches have been used to characterize the cytokine profile in RA. These have involved assays for cytokine biologic activity, based on various cytokine-dependent cell types and immunologic screening for cytokine protein (enzyme-linked immunosorbent assay, radioimmunoassay) (17,19–25). However, accurate interpretation of the results has been hindered by a number of confounding factors: the inherent lability of the cytokines, the presence of inhibitors, proteases, and inflammatory cell infiltrates, and the cross-reactivity between molecules of similar activity.

Earlier studies have addressed the potential of RA cells to be stimulated to produce specific cytokines (18,23), rather than on the inherent cytokine profile. Information regarding levels of cytokine gene expression in RA is limited (26–29). The sensitivity of the various assays used and the fact that stimulated and unfractionated synovial fluid (SF) and synovial tissue (ST) cells were used in one study (27) have limited the interpretation of results. Nevertheless, a pattern of a restricted cytokine profile is emerging. Elevated levels of the non-T cell–secreted cytokines GM-CSF (21), TNFα (30), TGFβ (18,31,32), and IL-1 (33,34) can be detected in RA synovium, implicating them in the development of membrane hyperplasia and pannus formation. There is accumulating evidence to suggest that IL-2 (21,29), IL-3 (21), IL-4 (18), and IFNγ (28,35) are undetectable or present in low levels in the RA synovium.

In the present study we examined cytokine gene expression by polymerase chain reaction (PCR) amplification of complementary DNA (cDNA) derived from specific messenger RNA (mRNA), using purified but uncultured RA T cells. This procedure allows quantitation of gene expression even when the number of gene transcripts is restricted to several copies per cell. The number of cells required is not a limiting factor in our protocol. Our results suggest that the RA synovial T cell, although phenotypically activated, is functionally inactive, at least with regard to the specific cytokines we examined, i.e., IL-2, IL-4, IL-6, and IFNγ. Since cell surface expression of the IL-2 receptor (IL-2R) is necessary for the proliferative response to IL-2 and is indicative of T cell activation (3), we concurrently examined IL-2R gene expression in the RA tissues.

Table 1. Clinical characteristics of the 37 rheumatoid arthritis patients studied*

Age, years	55.7 ± 12.4 (25–74)
Disease duration, years	14.8 ± 8.0 (3–27)
No. of actively inflamed joints	14.4 ± 10.9 (0–34)
No. of effusions	6.9 ± 5.4 (0–19)
Morning stiffness, minutes	236.3 ± 172.9 (0–480)
Lansbury index	58.5 ± 42.6 (0–118)
Hemoglobin, gm/dl	118.6 ± 16.4 (91–151)
ESR, mm/hour	47.9 ± 24.1 (22–120)

* Values are the mean ± SD (range). Number of actively inflamed joints defined by pain on motion, effusions, or tenderness. ESR = erythrocyte sedimentation rate.

PATIENTS AND METHODS

Patient selection. Thirty-seven patients (30 women, 7 men) with seropositive RA according to the American College of Rheumatology (formerly, the American Rheumatism Association) criteria (36), seen at the Rheumatic Disease Unit and Orthopaedic Service of the Wellesley Hospital, Toronto, were included in the study (Table 1). The control group (n = 5) consisted of age-matched healthy volunteers. Most patients were receiving a nonsteroidal antiinflammatory drug and a remittive agent. Of those receiving a remittive agent, 3 were taking either methotrexate or azathioprine. No patient was receiving >5 mg prednisone/day. Of the 17 patients from whom paired peripheral blood (PB)/SF samples were obtained, 3 were not receiving immunosuppressive therapy.

Preparation of ST, SF, tonsil tissue, and peripheral blood mononuclear cells. ST obtained at the time of surgical synovectomy was treated for 1.5 hours with DNase and collagenase in phosphate buffered saline (PBS) with 1% bovine serum albumin, at 37°C. Tonsil tissue was finely chopped in PBS. The resultant cell suspensions were strained through sterile gauze, then washed with PBS. SF was aspirated into heparinized syringes. SF and PB were diluted 1:1 with PBS.

Mononuclear cells were isolated from the different cell suspensions by density centrifugation on a Ficoll-Hypaque gradient at 2,400 revolutions per minute for 20

minutes, then washed twice with PBS in preparation for T cell separation. All cell preparations were processed immediately.

Mitogenic activation of lymphocytes. Lymphocytes from PB were resuspended in RPMI medium at a concentration of 1–1.2 × 10^6 cells/ml. Phytohemagglutinin (PHA; Difco, Detroit, MI) was added to a final concentration of 2 μg/ml. The cells were incubated for up to 48 hours at 37°C in 5% CO_2. T cells were separated at various time points.

T cell separation. T cells were separated using a standard rosetting protocol that routinely yielded >90% T cells. Briefly, following rosetting with AET-treated sheep erythrocytes, a T cell–enriched population was obtained by centrifugation on a Ficoll-Hypaque gradient at 3,000 rpm for 15 minutes. Sheep erythrocytes were removed by lysing in an NH_4Cl–Tris buffer at 37°C for 5 minutes. T cells were then washed twice with PBS, lysed in 4*M* guanidinium isothiocyanate, and either used immediately or stored at −70°C.

RNA isolation. RNA extraction from T cells has been described (37). Briefly, following lysis with guanidinium isothiocyanate, nucleic acid was extracted from cells with phenol/chloroform and the RNA precipitated with ethanol. Poly(A+) RNA was separated on oligo(dT) cellulose and precipitated for 2 hours at −70°C with 2*M* sodium acetate and ethanol.

Complementary DNA preparation. Poly(A+) RNA from 0.5 μg total RNA was reverse transcribed with 200 units Moloney murine leukemia virus reverse transcriptase, according to standard procedures (38). The cDNA (20 μl) was then used immediately in a PCR.

PCR amplification. Each PCR reaction was carried out in a total volume of 50 μl, containing the cDNA (20 μl), 50 m*M* KCl, 10 m*M* Tris, pH 8.3, 1.5 m*M* $MgCl_2$, 0.01% gelatin, 0.2 m*M* each of dATP, dCTP, dGTP, and dTTP, 2.5 units *Taq* polymerase, 0.4 μM of each specific primer (IL-2, IL-2R, IFNγ, IL-4, IL-6, β-actin; all from Clontech, Palo Alto, CA), and 10 μCi of ^{32}P-dATP. Amplification of individual cytokines was carried out in separate tubes. Each reaction tube was overlaid with 50 μl mineral oil. Because the cDNAs generated were derived from mRNA from a restricted pool of cells (T cells), we reduced the template concentration in our PCR reaction mix to 0.5 μg, compared with a concentration of 1.0 μg used generally. To ensure that amplification was complete, therefore, we proceeded with 40 cycles of PCR amplification, each cycle consisting of a denaturation step at 94°C for 1 minute, annealing at 58°C for 2 minutes, and polymerization at 72°C for 3 minutes. In the final cycle, polmerization was for 7 minutes.

Electrophoresis and autoradiography. Following PCR amplification, each sample was fractionated on a separate Sephadex G50 spin column to remove unincorporated radionucleotides, and 15 μl aliquots were electrophoresed on a 1% agarose gel at 55V for 2 hours. A *Hind* III–digested lambda marker was included in each run. The identity of the amplified products was confirmed by their specific respective sizes: IL-2 458 basepairs, IL-2R 398 bp, IL-4 456 bp, IL-6 628 bp, IFNγ 494 bp, and β-actin 1,126 bp. The agarose gels were soaked in 7% trichloroacetic acid for 30 minutes, then dried. The dried gel was then exposed to Kodak XAR-5 x-ray film (Eastman-Kodak, Rochester, NY). The size of the autoradiographic signal was quantitated using laser densitometry, and all values were normalized against the standard positive control signals.

Statistical analysis. Comparisons of levels of cytokine mRNA expression in paired PB and SF samples were assessed using paired *t*-test. Comparisons of patient and control cytokine mRNA expression were examined by the Wilcoxon rank sum test.

RESULTS

Cytokine production by activated PB T cells. Initial experiments compared the kinetics of induction and accumulation of cytokine mRNAs following PHA

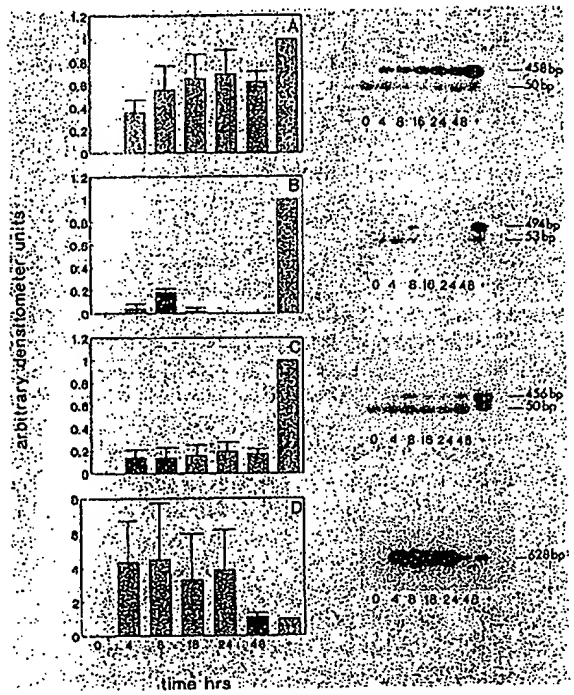

Figure 1. Kinetics of cytokine induction in phytohemagglutinin (PHA)–activated normal peripheral blood (PB) T cells. PB lymphocytes were activated with PHA for the times indicated, the T cells separated, and cytokine messenger RNA (mRNA) expression quantitated by polymerase chain reaction (PCR) amplification of complementary DNAs derived from isolated poly(A+) mRNA. A, Interleukin-2 (IL-2); B, interferon-γ; C, IL-4; D, IL-6. + designates the positive tonsil T cell control included in each PCR determination, normalized to a value of 1; levels of cytokine mRNA expression are quantitated relative to this value. Values are the mean and SD. There was variation among the different donor lymphocytes that were PHA activated, as indicated by the SD bars. Autoradiograms of the electrophoresed PCR products, each representing 1 of replicate experiments, are shown on the right. The upper band in the doublet depicts the presence of the specific cytokine, the lower band the primer–dimer complex.

Figure 2. Effects of tissue processing on mRNA integrity. Lymphocytes from the PB of a healthy donor were activated with PHA for 12 hours, then aliquoted for either immediate RNA extraction (A), DNase and collagenase treatment as described in Patients and Methods, followed by RNA extraction (B), Ficoll-Hypaque gradient separation followed by RNA extraction (C), or DNase and collagenase treatment followed by Ficoll-Hypaque gradient separation, then RNA extraction (D). Expression of IL-2, IL-6, and β-actin mRNA was examined by PCR amplification of complementary DNAs derived from the RNAs isolated at these different stages. See Figure 1 for definitions.

activation of PB from healthy donors. In a time course study, we quantitated expression of mRNA for IL-2, IFNγ, IL-4, and IL-6 in fractionated PB T cells, using the PCR protocol. The results of replicate experiments are shown in Figure 1. Randomly selected RNA samples were aliquoted, and then replicate samples were reverse transcribed to generate cDNA that was subsequently amplified by PCR. We recorded similar outcomes with regard to gene expression levels in replicate experiments (results not shown).

At time 0, unstimulated PB T cells had no detectable levels of IL-2, IFNγ, IL-4, or IL-6 mRNA. Whereas mRNA for IL-2, IL-4, and IL-6 was induced by 4 hours after PHA activation and remained elevated for 24–48 hours, IFNγ mRNA was transiently induced by 4 hours, peaked at about 8 hours, and diminished by 16 hours. The positive control included in each PCR procedure was a 48-hour PHA-activated tonsil T cell preparation. The PCR-amplified signal recorded for each cytokine in this preparation was normalized to a value of 1. IFNγ mRNA expression was detectable in the PHA-activated tonsil preparation at 48 hours. Although maximally activated PHA blasts served as our positive control throughout, activated cells from other inflammatory fluids would have been preferable, but this material was not readily available.

To examine the effect of tissue processing on levels of RNA expression, lymphocytes from the PB of a healthy donor were activated with PHA for 12 hours, then aliquoted for either immediate RNA extraction (Figure 2, lanes A), DNase and collagenase treatment as described in Patients and Methods, followed by RNA extraction (Figure 2, lanes B), Ficoll-Hypaque gradient separation followed by RNA extraction (Figure 2, lanes C), or DNase and collagenase treatment followed by Ficoll-Hypaque gradient separation, then RNA extraction (Figure 2, lanes D). Tissue processing did result in a reduction in viable cell yield at the different stages: collagenase/DNase treatment 18% loss, Ficoll-Hypaque separation 5% loss, and collagenase/DNase treatment followed by Ficoll-Hypaque separation 20% loss. However, 3–5 μg total RNA/10^6 cells was consistently recovered from the resultant viable cells at each stage in the purification procedure.

To ensure that the integrity of specific cytokine RNA species isolated at each stage (Figure 2, lanes A–D as described above) was not affected by our purification process, we examined IL-2, IL-6, and β-actin mRNA expression by PCR amplification of cDNAs derived from the RNAs isolated at these different stages. As seen in Figure 2, no variability in levels of mRNA expression for IL-2, IL-6, or β-actin was observed among the samples collected at the different stages in the purification procedure. We therefore concluded that the process per se does not affect the outcome with regard to RNA expression levels.

Cytokine mRNA expression in paired PB and SF T cell samples from patients with RA. In subsequent experiments we examined levels of mRNA expression for IL-2, IL-2R, and IFNγ in 17 paired RA PB and SF T cell preparations. The results are shown in Figure 3. Similar outcomes with regard to gene expression levels were recorded for randomly selected patient samples that were analyzed in replicate during different "test" runs (data not shown). We included the positive PHA-activated tonsil T cell control for each

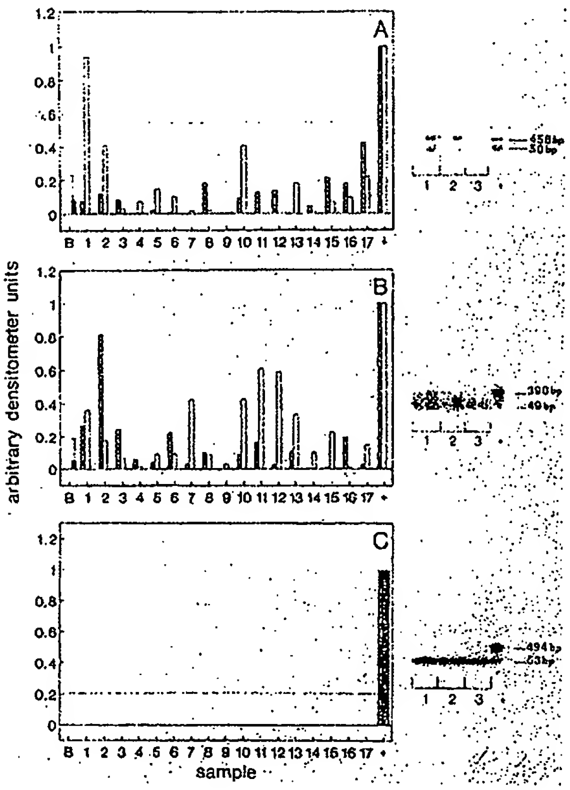

Figure 3. Quantitation of cytokine gene expression in matched PB (hatched bars) and synovial fluid (SF) (open bars) T cell samples from patients with rheumatoid arthritis. Seventeen pairs of samples were analyzed. Cytokine mRNA expression was quantitated by PCR amplification of complementary DNAs derived from isolated poly(A+) mRNA. A, IL-2; B, IL-2 receptor; C, interferon-γ. All values are quantitated relative to the positive tonsil T cell control (+) included in each PCR determination, normalized to a value of 1. B = the baseline level of mRNA expression determined from unstimulated PB T cells from healthy donors (mean and SD of 5 different samples). Horizontal dotted line in A and C represents the maximum value obtained when normal PB lymphocytes are stimulated with PHA, as in Figure 1. Representative autoradiograms of the electrophoresed PCR products from paired PB and SF samples 1, 2, and 3 are shown on the right; in each lane, the PB sample is on the left and the SF sample on the right. See Figure 1 for other definitions.

cytokine in each of the PCR amplifications, as well as a baseline control determined from unstimulated PB T cells obtained from 5 healthy donors.

Of the 17 PB T cell samples analyzed for IL-2 mRNA, only 1 sample (17) exhibited mRNA levels more than 2 standard deviations above the mean level in healthy controls (Figure 3A). Of the 17 matched SF T cell samples analyzed, only 3 samples (1, 2, and 10) had mRNA expression levels more than 2 standard deviations above the mean in the baseline controls. The mean level of IL-2 mRNA expression in PB did not differ significantly from the mean in SF ($P = 0.2$), but IL-2 mRNA expression in individual PB samples did not correlate with that in matched SF samples.

Figure 3B shows similar analyses for IL-2R. Five PB samples (samples 1, 2, 3, 6, and 16) exhibited elevated mRNA expression for IL-2R. Seven SF samples (samples 1, 7, 10, 11, 12, 13, and 15) exhibited elevated IL-2R mRNA levels. Again, IL-2R mRNA expression in PB T cell samples did not correlate with expression in matched SF T cell samples. We were unable to detect any IFNγ mRNA in any of the 17 PB and SF T cell preparations, even when a 3-fold increase in the amount of poly(A+) RNA template was used in each PCR (Figure 3C).

We also compared mean levels of cytokine mRNA expression in PB and SF from the RA group versus those in PB from the control group. Mean IL-2R mRNA expression in RA SF was significantly elevated relative to that observed in normal PB ($P < 0.01$). However, there was no significant difference in IL-2R mRNA levels in PB between RA patients and control subjects ($P > 0.05$). Additionally, mean IL-2 mRNA expression in RA PB or SF did not differ from levels in normal PB ($P > 0.05$).

Cytokine mRNA expression in RA ST T cells. Since our studies showed consistently low levels or absence of T cell mRNA expression for IL-2, IL-2R, and IFNγ in the PB and SF samples from patients with RA, we next examined mRNA expression for these cytokines in RA ST T cell samples. The results are presented in Figure 4. Randomly selected patient samples were again analyzed repeatedly in different "test" runs, which ensured reproducibility of outcomes (results not shown). Matched PB and SF samples were not available for these ST samples. All values were normalized against the corresponding PHA-activated (48-hour) tonsil T cell preparations included in each PCR.

Of the 12 RA ST T cell preparations examined for IL-2 mRNA, only 1 (sample 25) showed mRNA expression more than 2 standard deviations higher than the mean in the normal baseline control (Figure 4A). Moreover, the mean level of IL-2 mRNA expression in RA ST was significantly lower than the level in normal PB ($P < 0.02$). Of the 6 RA ST T cell preparations examined for IL-2R mRNA expression (corresponding to samples 25–31 analyzed for IL-2 expression), none showed expression above that of the

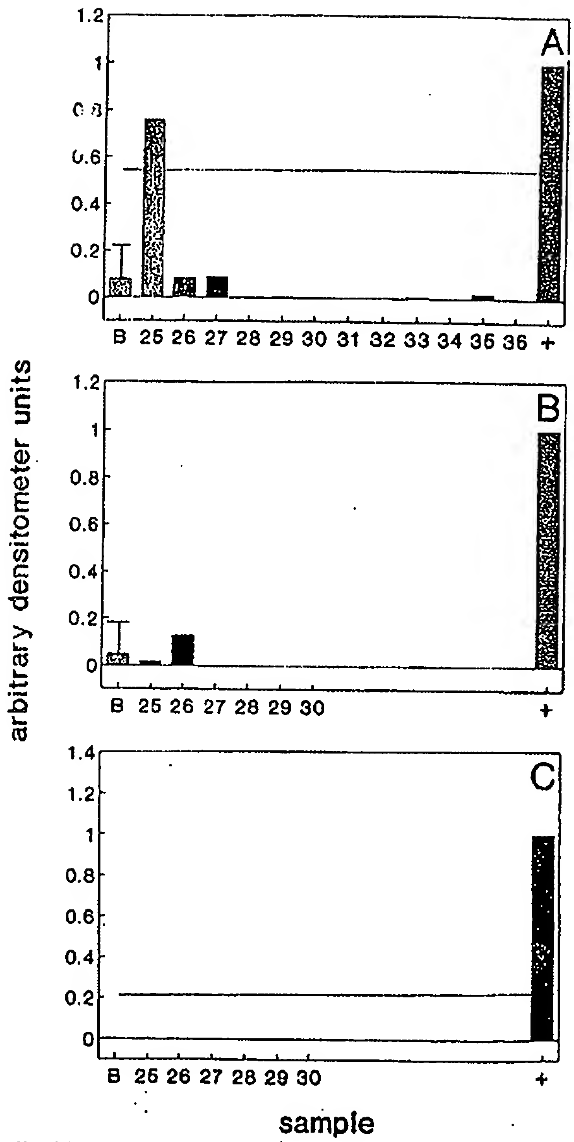

Figure 4. Quantitation of cytokine gene expression in rheumatoid arthritis synovial tissue (RA ST) T cells. A total of 12 RA ST samples were analyzed, all for IL-2 mRNA expression (A) and 6 each for IL-2 receptor mRNA expression and interferon-γ mRNA expression (B and C, respectively). Cytokine mRNA expression was quantitated by PCR amplification of complementary DNAs derived from isolated poly(A+) mRNA. All values are quantitated relative to the positive tonsil T cell control (+) included in each PCR determination. B = the baseline level of mRNA expression determined from unstimulated PB T cells from healthy donors (mean and SD of 5 different samples). The 12 tissues analyzed do not correspond to any of the patient samples in Figure 2. Horizontal line represents the maximum value obtained when normal lymphocytes are stimulated with PHA, as in Figure 1. See Figure 1 for other definitions.

baseline control (Figure 4B). Again, the mean level of IL-2R mRNA expression in RA ST was lower than that detected in normal PB ($P < 0.05$). In none of the 6 RA ST T cell preparations were we able to detect any mRNA for IFNγ, even when a 3-fold increase in the amount of poly(A+) RNA template was used in each PCR (Figure 4C).

Additionally, when we examined RA SF and ST T cell preparations, randomly selected from the patient samples, for IL-4 and IL-6 mRNA expression (Figure 5), the data revealed a paucity of these cytokines: IL-4 gene expression levels were consistently low in SF, with only 1 of 11 samples tested (sample 20) exhibiting RNA expression more than 2 standard deviations above the baseline control mean. None of the 6 ST samples tested showed IL-4 gene expression more than 2 standard deviations above that found in baseline controls. For IL-6 gene expression, 3 of the 7 SF samples tested (samples 2, 12, and 13) and 2 of the 8 ST samples tested (samples 26 and 35) exhibited mRNA levels more than 2 standard deviations above the mean in baseline controls.

β-actin mRNA expression. To determine whether the poor gene expression observed in the RA T cells is restricted to cytokine gene expression or reflects an overall down-regulation of mRNA expression, we examined levels of expression of the cell cycle invariant gene, β-actin, in normal PB T cells, and in RA SF and ST T cells. The results are shown in Figure 6. In normal PB T cells and the randomly selected RA SF T cell samples, (samples 7 and 12), β-actin mRNA expression was detected. Six ST T cell samples (samples 25, 31, 33, 34, 35, and 36) were examined for β-actin mRNA expression. All had clearly detectable β-actin expression, which was elevated above that observed in the normal PB T cells. It is noteworthy that in 3 of these 6 samples (samples 31, 34, and 36), there was no detectable IL-2, IL-4, or IFNγ mRNA.

DISCUSSION

Persistent inflammatory processes play a critical role in the pathogenesis of rheumatoid arthritis. An extensive network of interactive cytokines regulates inflammatory reactions, yet the mechanisms underlying their mediation of inflammatory reactions in RA are unclear. Accumulated evidence from many studies indicates that rheumatoid synovitis is characterized by significant T cell infiltration and that a large proportion of synovial T cells express HLA class II antigens, a T cell activation marker, and very late activation antigens, such as VLA-1 (39,40). The objective of the

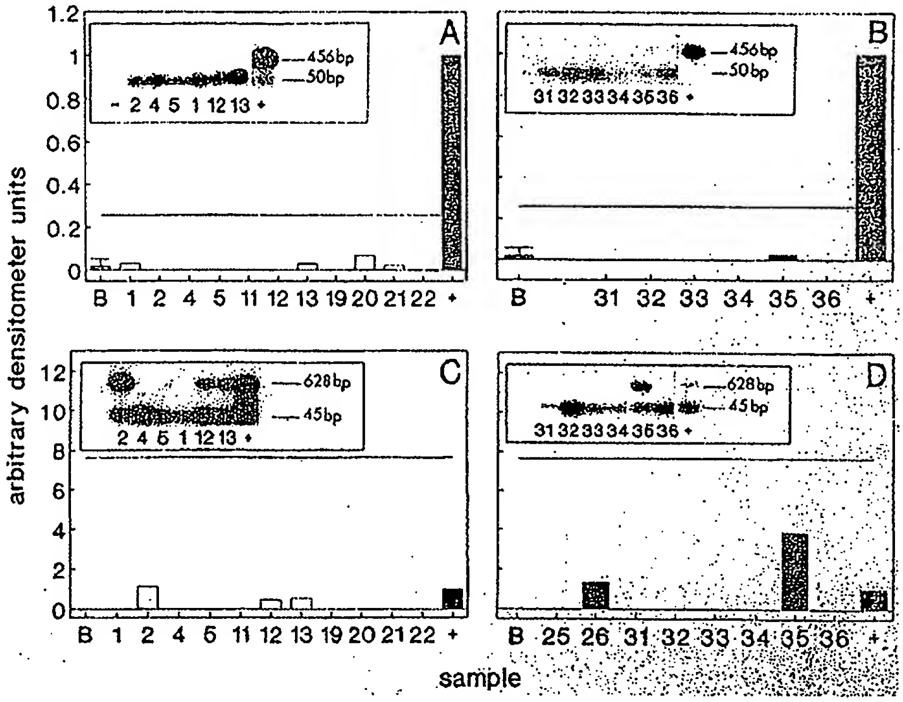

Figure 5. Quantitation of IL-4 and IL-6 mRNA expression in rheumatoid arthritis synovial fluid (RA SF) and RA synovial tissue (RA ST) T cells. Randomly selected RA SF and RA ST samples were assessed for IL-4 and IL-6 mRNA expression by PCR amplification of complementary DNAs derived from isolated poly(A+) mRNA. A, SF IL-4; B, ST IL-4; C, SF IL-6; D, ST IL-6. SF sample numbers correspond to the sample numbers in Figure 3; and ST sample numbers to the sample numbers in Figure 4. All values are quantitated relative to the positive tonsil T cell control (+) included in each PCR determination. B = the baseline level of mRNA expression determined from unstimulated PB T cells from healthy donors (mean and SD of 5 different samples). Horizontal line represents the maximum value obtained when normal PB lymphocytes are stimulated with PHA, as in Figure 1. Representative autoradiograms of the electrophoresed PCR products are shown. See Figure 1 for other definitions.

present study was to examine the cytokine profile in RA T cells, and to determine the nature and extent of cytokine release in phenotypically activated RA T cells.

At the outset we chose to examine expression of mRNA for IL-2, IL-2R, IL-4, IL-6, and IFNγ. We employed a PCR amplification procedure to quantitate mRNA expression, since the sensitivity of this technology would allow detection of few copies of mRNA

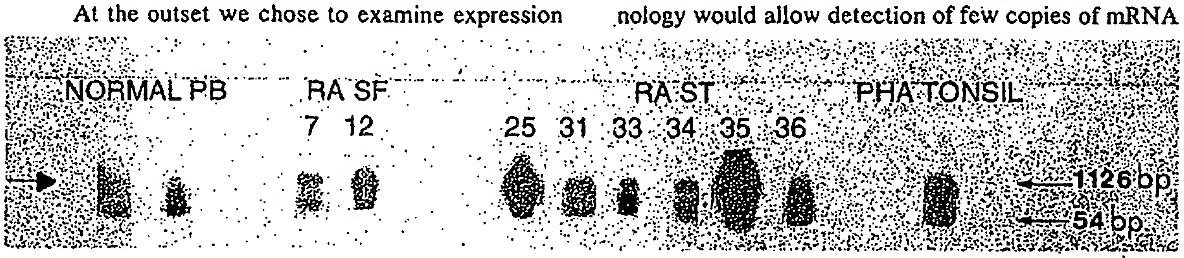

Figure 6. Expression of β-actin mRNA in rheumatoid arthritis synovial fluid (RA SF) and RA synovial tissue (RA ST) T cells. Randomly selected RA SF and RA ST samples were assessed for β-actin mRNA expression by PCR amplification of complementary DNAs derived from isolated poly(A+) mRNA. β-actin expression in PB T cells from normal donors was also assessed. The positive tonsil T cell control was included in each PCR determination. Autoradiograms of the electrophoresed PCR products are shown. The upper band is the amplified 1,126-bp β-actin complementary DNA. RA SF and RA ST sample numbers correspond to the sample numbers in previous Figures 3–5. See Figure 1 for other definitions.

in low numbers of cells. Results were standardized to a positive control included in all reactions. The same, single pool of PHA-activated tonsil cells was used as the positive control in all of our experiments, since PHA activation is generally accepted to reflect the maximum inducible levels of cytokine expression in T cells. Our data indicated that the process we used for purification and extraction of RNA did not affect the integrity of the RNA isolated for analysis.

Considerable variation was seen in the expression levels for the different cytokines among the different patient tissues, though levels were consistently low. We showed that the paucity of cytokine mRNA expression in RA T cells is restricted and is not a general down-regulation phenomenon, since β-actin expression levels were unaffected. Individual patients exhibited variation in levels of expression of different cytokines. Moreover, we were unable to correlate levels of expression detected for any of the cytokines between matched PB and SF samples from individual patients. This lack of correlation is to be expected when one considers that the immediate environment of the RA synovium is infiltrated with reactive cells associated with host defense, inflammation, and tissue repair, which induce an array of cytokines that would influence the cytokine release of the synovial T cell, specifically.

We had anticipated that examination of SF for evidence of cytokine production by surrounding tissues would accurately reflect the immunologic activity within the synovium. Although matched SF and ST samples were not available, the data suggest that the trend of impaired cytokine production evident in the ST samples was generally reflected in the SF samples as well. Taken together, these results indicate that RA T cells, specifically ST T cells, are functionally impaired with respect to cytokine release, particularly of IFNγ.

The low levels of cytokine mRNA expression that we attribute to the T cells in these studies may actually originate elsewhere. Some consideration must be given to the low-level monocyte contamination in fractionated samples (41–44), which could account for the IL-2R and IL-6 expression levels we observed. Indeed, the kinetics of IL-6 induction shown in Figure 1 are characteristic of monocytes. This interpretation would further support our conclusion that there is significant restricted cytokine gene expression in RA T cells.

One important finding was the failure to detect any IFNγ mRNA in any of the patient samples we examined. Time course studies suggested that IFNγ mRNA is transiently induced on activation, at least in PB T cells, although PHA-activated tonsil T cells exhibited detectable IFNγ mRNA as late as 48 hours after activation. Although the late-activation state of many of the RA T cells may account for some reduction in the IFNγ mRNA present, the patient samples were asynchronous with respect to activation, and thus earlier-activated T cells would also have been present in the sample pool. Indeed, IL-2R mRNA expression is frequently used as an indicator of early T cell activation, and since we were able to show elevated levels of IL-2R in 6 RA SF and 2 RA PB samples, we expected that the PCR procedure employed should have been sensitive enough to detect even a few copies of IFNγ mRNA in these RA samples. Since none were detected, we conclude that there is a profound absence of IFNγ in RA T cells.

The low levels of T cell–derived cytokines such as IL-2, IL-4, and IL-6, and the significant absence of IFNγ, in RA PB, SF, and ST, despite the presence of T cells that are phenotypically activated, suggest that any T cell immunoregulatory functions necessary to prevent self-reactivity and the destruction of articular tissues by cytokine-responsive mesenchymal cells are either diminished or absent. The mechanism(s) accounting for the low levels of T cell–derived cytokines in RA SF and ST cells that are phenotypically activated remains unclear.

In vivo, homeostatic mechanisms regulating immune function are subserved in part by the antagonism between different T cell–derived and non–T cell–derived cytokines. IFNγ and TNFα are two such cytokines that are mutually antagonistic (2). It is of significance, therefore, that elevated levels of TNFα have been reported in RA (30). Production of IL-2, IL-4, and IL-6 is inhibited by TGFβ (16,17), and elevated levels of TGFβ have been reported in RA (18,31,32). Moreover, TGFβ has been implicated in the inhibitory influence of RA SF upon lymphocyte proliferation. These data suggest that defective T cell function in RA may arise from disruption of homeostatic regulatory mechanisms, leading to excessive production of synovial cell–derived cytokines, which down-regulate synovial T cells. The consequence of such a regulatory imbalance may permit the persistence of a putative infectious agent, or impair antigen-specific T cell suppressive influences within the synovial environment. Further investigation of the antagonism between the proinflammatory cytokines that are abundant, and the specific T cell–secreted cytokines that are restricted, in the RA synovium

should provide information relating to the therapeutic potential of specific T cell–induced cytokines in RA.

ACKNOWLEDGMENT

The authors are grateful to K. M. Snow for her assistance in retrieving and collating patient data that were used in these studies.

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